time microscopy along with ISH and IHC to assess orbital development following late inhibition of RA synthesis or morpholino knockdown of Twist1a. *Results*: We show that in zebrafish, the neural crest-derived periocular mesenchyme is a key regulator of EOM organization, and that RA is a mediator of this activity. EOMs differentiate appropriately, but the exact anatomic localization of EOMs around the eye is disrupted. We further show that we can suppress the effects of DEAB, an inhibitor of RA synthesis, using exogenously applied RA, and that the EOMs and the developing jaw are differentially sensitive to RA concentrations. Interestingly, morpholino knockdown of Twist1a also affects EOM organization, mimicking in zebrafish a key aspect of a human disorder. We will describe the model and how our results shed important light on processes that underlie orbital disorders and anatomic strabismus. *Conclusion*: Zebrafish orbital development reveals a dependence on RA signaling that is very similar to data from existing mammalian models. We further identify a role for Twist1a in periocular development and EOM organization.

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Program/Abstract # 202
Lens pit morphogenesis requires epithelial cell shape changes mediated by Shroom3
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A number of signaling pathways and transcription factors required for lens development and morphogenesis have been identified. However, the mechanisms underlying morphogenetic movements of early lens formation remain largely uncharacterized. During lens formation, the presumptive lens ectoderm invaginates in a basal direction concomitantly with the closely opposed optic vesicle forming the lens pit and optic cup. Individual cells within the lens pit change from a cylindrical shape to a conical shape and it is hypothesized that this cell shape change is a driving force of lens pit invagination. The cytoskeletal protein Shroom3, an actin binding protein known to be required for neural tube morphogenesis, can cause this type of cell shape change by inducing apical constriction of epithelial cells. Expression analysis during lens invagination revealed that Shroom3 is apically localized in cells within the lens pit. To determine if Shroom3 is required for the cell shape change during lens pit invagination Shroom3 mutant embryos were examined. Apical constriction is inhibited in lens pit cells of Shroom3 mutant mouse embryos. The lens pits are also smaller, misshapen, and display a redistribution of F-actin and non-muscle myosin. Analysis of Pax6 mutant embryos demonstrated that Shroom3 expression depends on the initiation of the lens induction pathway. Deletion of a domain within Shroom3 was also performed to analyze its role in apical constriction and apical actin accumulation. Together, these data demonstrate a requirement for Shroom3 during lens pit invagination and provide novel mechanistic clues into the function of Shroom3.

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Program/Abstract # 203
Myosin-X is critical for the migratory ability of *Xenopus* cranial neural crest cells
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The neural crest is a highly migratory cell population, unique to vertebrates, that forms much of the cranial skeleton and peripheral nervous system. To understand the cell biological basis underlying their behavior, we have explored the functional role of myosin-X (Myo10), an unconventional myosin, a novel factor that we show is required for neural crest migration. Myo10 is highly expressed in both premigratory and migrating cranial neural crest (CNC) cells in *Xenopus* embryos. Disrupting Myo10 expression using antisense morpholino oligonucleotides leads to delayed neural crest induction and impaired neural crest migration. Both in vitro explant cultures and in vivo grafting experiments suggest that Myo10 is required for CNC migration. Myo10-depleted CNC cells migrate a shorter distance and fail to segregate into distinct migratory streams. Finally, cell dissociation-reaggregation assays suggest that Myo10 may control CNC cell adhesion. Thus, these results reveal a requirement for Myo10 in normal neural crest migration and suggest a link to cellcell and cellmatrix interactions.

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Program/Abstract # 204
Twist-family member interactions regulate cardiac neural crest morphogenesis
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Murine tissue-specific gene ablation models have considerably furthered our understanding of the roles various genes play in cardiac neural crest cell (cNCC) migration, adhesion, and differentiation and congenital heart disease. Twist-family basic helixloophelix (bHLH) transcription factors, including Twist1, Hand1 and Hand2, molecularly interact to form uniquely functional transcriptional complexes and are dynamically expressed in the cNCCs of the developing cardiac outflow tract (OFT) and pharyngeal arches (PAs). During OFT morphogenesis, Twist1 regulates cNCC emigration, maturation, and, cell adhesion. *Twist1*$^{-/-}$ mice display cNCC abnormalities in the dorsal neural tube, PAs, and a subpopulation of OFT cNCCs expressing *Hand1* and *Hand2*. Relative gene dosage of *Twist1* and *Hand2* within the developing limb is critical, as genetic disruption of the balance of these two factors causes polydactyly. The unique cNCC phenotypes in the aorticopulmonary (AP) septum of *Twist1*$^{-/-}$;*Hand2*$^{+/-}$ mutants and in the OFT cushions of *Twist1*$^{-/-}$;*Hand2*$^{fx/-}$;*Wnt1-Cre* mutants indicate additional genetic interactions between *Twist1* and *Hand2*. Current studies employing systemic and NCC-specific inactivation of these factors will define cooperative and/or antagonistic *Twist1* and *Hand2* functions within cNCCs. Together, these data will broaden out understanding of bHLH factor interaction and transcriptional regulation of cNCC contribution to specific OFT structures, and define, in terms of gene transcription and both cellular and genetic interaction, the role of the bHLH code in OFT development.

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Program/Abstract # 205
MicroRNAs in cardiac neural crest are critical for cardiac outflow tract patterning
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